WHAT IS CLAIMED IS:

1. An orthogonal modulator of a mutant GTPase, which orthogonal modulator modulates an activity of a mutant GTPase but does not substantially modulate an activity of a corresponding wild-type GTPase.

2. The othogonal GTPase modulator of claim 1, wherein the GTPase modulator comprises a guanine ring modified at one or more of a C-6, N-7 and/or N-9 position.

3. The othogonal GTPase modulator of claim 1, wherein the GTPase modulator comprises a guanosine modified at a C-2, C-6 and/or N-7 position.

4. The othogonal GTPase modulator of claim 1, wherein the GTPase modulator comprises a structure selected from the group consisting of: a guanosine triphosphate modified at a C-6 and/or N-7 position; a guanosine diphosphate modified at a C-6 and/or N-7 position; and a guanosine monophosphate modified at a C-6 and/or N-7 position.

5. The othogonal GTPase modulator of claim 1, wherein the GTPase modulator comprises a cell permeable compound.

6. The othagonal GTPase modulator of claim 1, wherein the GTPase modulator comprises:

a) a sulfhydryl group that forms a disulfide linkage with a sulfhydryl group on N116C of the mutant GTPase, or

b) an electrophilic group that forms a covalent bond with a sulfhydryl group on N116C of the mutant GTPase.

7. The othogonal GTPase modulator of claim 1, wherein the GTPase modulator comprises:

a) a sulfhydryl group that forms a disulfide linkage with a sulfhydryl group on T144C, or

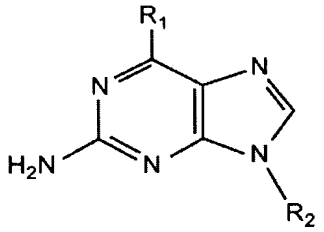
b) an electrophilic group that forms a covalent bond with a sulfhydryl group on T144C.

8. The othogonal GTPase modulator of claim 1, wherein the GTPase modulator comprises:

a) a sulfhydryl group that forms a disulfide linkage with a sulfhydryl group on L19C, or

b) an electrophilic group that forms a covalent bond with a sulfhydryl group on L19C.

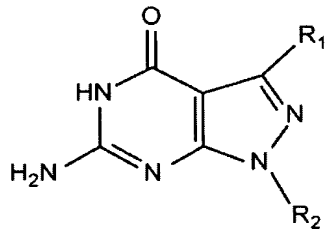
9. The othogonal GTPase modulator of claim 1, wherein the GTPase modulator comprises the structure:




wherein R1 is selected from the group consisting of: O-benzyl, O-$(CH_2)_2$phenyl, NH-benzyl, NH-$(CH_2)_2$phenyl, O-$CH_2$*tert*-butyl, O-isopropyl, O-$(CH_2)_2$naphthyl, O-$(CH_2)_3$cyclohexyl, O-$(CH_2)_2$cyclohexyl, O-$CH_2$ cyclohexyl, and N-isobutyl; and

wherein R2 comprises a benzyl group or a methyl-*tert*-butyl group.

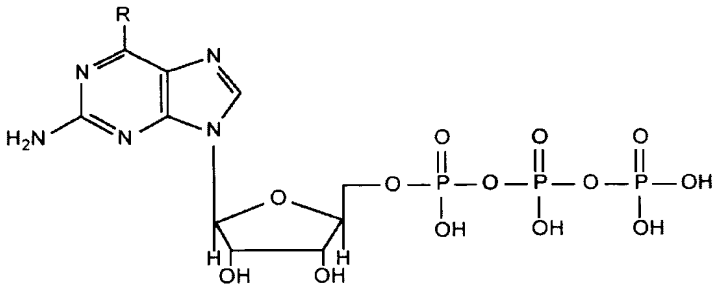
10. The othogonal GTPase modulator of claim 1, wherein the GTPase modulator comprises the structure:




wherein R1 is selected from the group consisting of phenyl, $CH_2$-naphthyl, and cyclohexyl; and

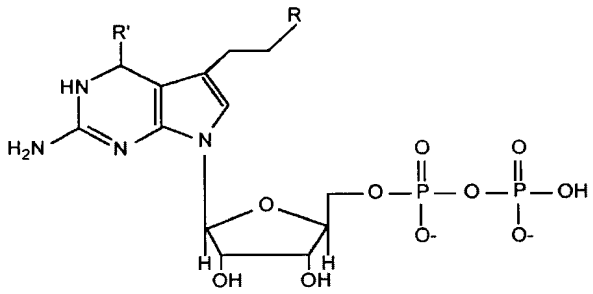
wherein R2 is selected from the group consisting of benzyl and methyl-*tert*-butyl.

11. The othogonal GTPase modulator of claim 1, wherein the GTPase modulator comprises the structure:



wherein R is selected from the group consisting of NH-benzyl, O-*iso*butyl, NH-(2-phenyl)ethyl, NH-*iso*butyl, O-2-propene, O-methyl, and thiol.

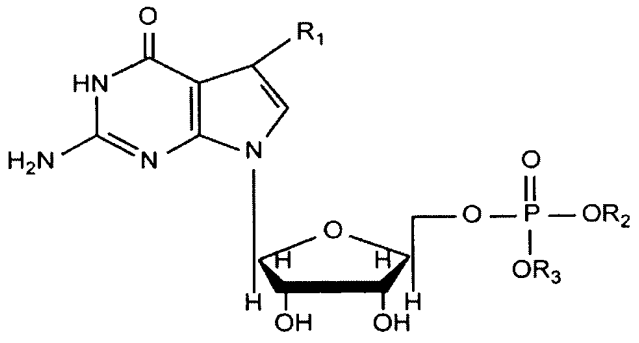
12. The othogonal GTPase modulator of claim 1, wherein the GTPase modulator comprises the structure:



wherein R is selected from the group consisting of: *iso*propyl, *tert*-butyl, cyclohexyl, phenyl, 4-fluorophenyl, benzyl, and 2-naphthyl; and

wherein R' comprises a ketone or $NH_2$.

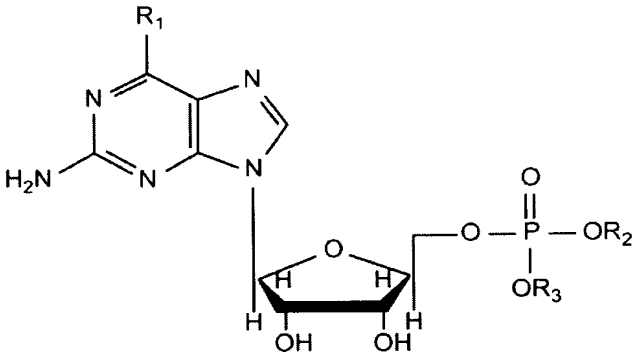
13. The othogonal GTPase modulator of claim 1, wherein the GTPase modulator comprises the structure:



wherein R1 comprises thiol, $CH_2SH$, $-CH_2CH_2SH$, $-CH_2CH_2CH_2SH$, $-CH_2CH_2CH_2CH_2SH$, $-CH_2CH_2NHCOCH_2CH_2$-N-maleimide, $-CH_2CH_2NHCOCH{=}CH_2$, $-CH_2CH_2NHCO$-(4-fluorosulfonyl)phenyl, or 4-fluorophenyl; and

wherein R2 and R3 are independently selected from the group consisting of -$OCH_2OCH_2CH_3$, -$OCH_2CH_2SCOCH_3$, and -$OCH_2CH_2CH_2CH_2CH_2SCOCH_3$.

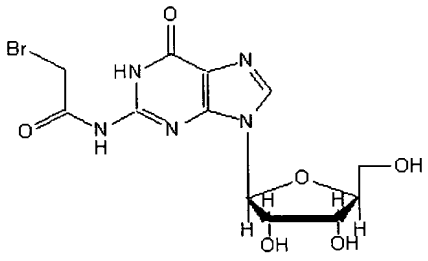
14. The othogonal GTPase modulator of claim 1, wherein the GTPase modulator comprises the structure:



wherein R1 comprises thiol, -$OCH_2CH_2SH$, -$OCH_2CH_2CH_2SH$, -$OCH_2CH_2CH_2CH_2SH$, -$OCH_2CH_2NHCOCH_2CH_2$-N-maleimide, -$OCH_2CH_2NHCOCH{=}CH_2$, -$OCH_2CH_2NHCO$-(4-fluorosulfonyl)phenyl, $OCH_2CH_2$-(3-methyl)maleimide, -$OCH_2CH_2$-(3, 4-dimethyl)maleimide, or $OCH_2CH_2CH_2$-(3, 4-dimethyl)maleimide; and

wherein R2 and R3 are independently selected from the group consisting of -$OCH_2OCH_2CH_3$, -$OCH_2CH_2SCOCH_3$, and -$OCH_2CH_2CH_2CH_2CH_2SCOCH_3$.

15. The othogonal GTPase modulator of claim 1, wherein the GTPase modulator comprises the structure:



16. The othogonal GTPase modulator of claim 1, wherein the GTPase modulator comprises a substituted guanine ring having R1 attached at a C6 position, R2 attached at a C-7 position, and R3 attached at an N9 position;

wherein R1 is selected from the group consisting of a ketone, a thiol, a methyl thiol, an ethyl thiol, a propyl thiol, a butyl thiol, an O-thiol, an O-ethylthiol, an O-propylthiol, an O-butylthiol, an O-propyl group, an O-isopropyl group, an O-isobutyl group, an O-*sec*-butyl group an O-*tert*-butyl group, an O-(2,2-dimethyl)propyl group, an O-cyclohexyl group, an O-methylcyclohexyl group, an O-(2-cyclohexyl)ethyl group, an O-(3-cyclohexyl)propyl group, an O-phenyl group, an O-benzyl group, an O a (2-phenyl)ethyl group, an O-[2-(1-naphthyl)]ethyl group, an O-[2-(2-naphthyl)]ethyl group, an N-propyl group, an N-isopropyl group, an N-isobutyl group, an N-*sec*-butyl group, an N-*tert*-butyl group, an N-(2,2-dimethyl)propyl group, an N-cyclohexyl group, an N-methylcyclohexyl group, an N-(2-cyclohexyl)ethyl group, an N-(3-cyclohexyl)propyl group, an N-phenyl group, an N-benzyl group, an N-(2-phenyl)ethyl group, an N-[2-(1-naphthyl)]ethyl group, an N-[2-(2-naphthyl)]ethyl group, an O-[(3-maleimido)propylamido]ethyl group, an O-[(3-methyl)maleimido]ethyl group, an O-[(3, 4-dimethyl)maleimido]ethyl group, an O-[(3, 4-dimethyl)maleimido]propyl group, an-(2-*N*-acrylamido)ethyl group, an O-(n-*N*-acrylamido)alkyl group, an alkyl halide group; a (2-phenyl)ethyl group, a [2-(1-naphthyl)ethyl group, and a [2-(2-naphthyl)ethyl group

wherein R2 is selected from the group consisting of a ketone, a thiol, a methyl thiol, an ethyl thiol, a propyl thiol, a butyl thiol, an *n*-propyl group, an isopropyl group, an isobutyl group, a *sec*-butyl group, a *tert*-butyl group, a (2,2-dimethyl)propyl group, a cyclohexyl group, a methylcyclohexyl group, a (2-cyclohexyl)ethyl group, a (3-cyclohexyl)propyl group, a phenyl group, a benzyl group, a (2-phenyl)ethyl group, a pyridine group, a 3-pyrroline group, a [2-(1-naphthyl)]ethyl group, and a [2-(2-naphthyl)]ethyl group; and

wherein R3 comprises a hydrogen, a ribose sugar, a monophosphorylated ribose, a diphosphorylated ribose, a triphosphorylated ribose, a ribose comprising one or more caged phosphate groups, a benzyl group or a methyl-*tert*-butyl group.

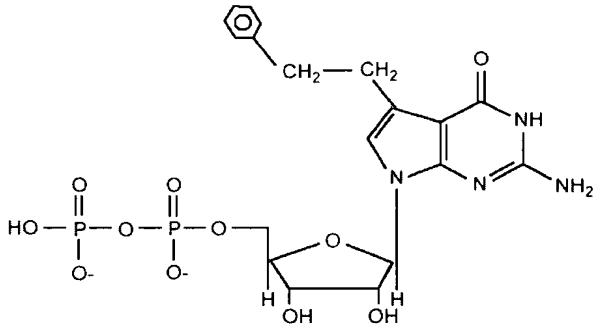
17. The othogonal GTPase modulator of claim 1, wherein the GTPase modulator comprises a substituted guanine ring having a first substituent attached at a C6 position, a second substituent attached at a C-7 position, and a third substituent attached at an N9 position, and wherein at least one of the substituents comprises an electrophilic moiety.

18. The othogonal GTPase modulator of claim 17, wherein the electrophilic moiety comprises a [(3-maleimido)propylamido]ethyl group, a [(3-methyl)maleimido]ethyl group,

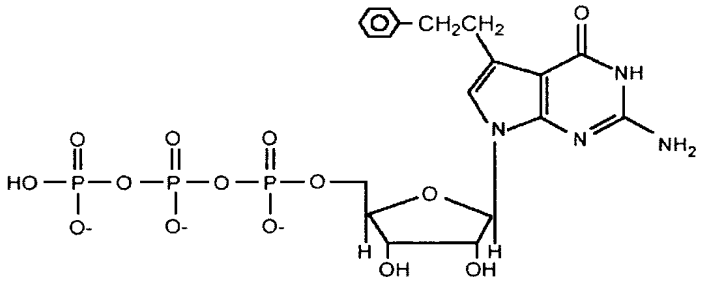
a [(3, 4-dimethyl)maleimido]ethyl group, a [(3, 4-dimethyl)maleimido]propyl group, a (2-*N*-acrylamido)ethyl group, a (n-*N*-acrylamido)alkyl group, a thiol group, an alkyl halide or an alkyl thiol group.

19. The othogonal GTPase modulator of claim 1, wherein the electrophilic moiety comprises an O-linked substituent or an N-linked substituent.

20. The othogonal GTPase modulator of claim 1, wherein the GTPase modulator is:



21. The othogonal GTPase modulator of claim 1, wherein the GTPase modulator is:



22. A mutant GTPase comprising a non-native amino acid at one or more amino acid positions that correspond to L19, F28, N116, K117 and T144 of H-Ras, which mutant GTPase binds to a GTPase modulator that modulates the mutant GTPase but does not substantially modulate a corresponding wild-type GTPase.

23. The mutant GTPase of claim 22, wherein the GTPase modulator comprises an inhibitor of GTPase activity.

24. The mutant GTPase of claim 22, wherein the GTPase modulator comprises an activator of GTPase activity.

25. The mutant GTPase of claim 22, wherein the non-native amino acid is selected from the group consisting of N116A, N116G and N116C.

26. The mutant GTPase of claim 22, wherein the GTPase modulator comprises a sulfhydryl group that forms a disulfide linkage with a sulfhydryl cysteine sidechain on the mutant GTPase.

27. The mutant GTPase of claim 26, wherein the mutant GTPase comprises N116C or T144C.

28. The mutant GTPase of claim 22, wherein the GTPase modulator comprises an electrophilic group that forms a covalent bond with an amino acid sidechain on the mutant GTPase.

29. The mutant GTPase of claim 28, wherein the mutant GTPase comprises N116C or T144C.

30. The mutant GTPase of claim 22, wherein the non-native amino acid is selected from the group consisting of L19A, L19G, and L19C.

31. The mutant GTPase of claim 22, wherein the mutant GTPase comprises a first non-native amino acid at a position that corresponds to N116 of H-Ras and a second non-native amino acid at a position that corresponds to L19 of H-Ras.

32. The mutant GTPase of claim 31, wherein the first non-native amino acid is N116A or N116G and the second non-native amino acid is L19A, L19G, or L19C.

33. The mutant GTPase of claim 32, wherein the first non-native amino acid is N116A or N116G and the second non-native amino acid is L19C.

34. The mutant GTPase of claim 22, wherein the GTPase is a Ras GTPase.

35. The mutant GTPase of claim 34, wherein the mutant GTPase comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 2 to SEQ ID NO: 17.

36. The mutant GTPase of claim 35, wherein the mutant GTPase is encoded by a polynucleotide that comprises a nucleotide sequence selected from the group consisting of SEQ ID NO: 19 to SEQ ID NO: 34.

37. The mutant GTPase of claim 22, wherein the GTPase modulator comprises a structure selected from the group consisting of: a guanine ring modified at one or more of a

C-6, N-7 and/or N-9 position; a guanosine triphosphate modified at a C-6 and/or N-7 position; a guanosine diphosphate modified at a C-6 and/or N-7 position; a guanosine monophosphate modified at a C-6 and/or N-7 position; and a guanosine modified at a C-2, C-6 and/or N-7 position.

38. The mutant GTPase of claim 22, wherein the GTPase modulator comprises a cell permeable compound.

39. The mutant GTPase of claim 22, wherein the GTPase modulator comprises an orthogonal GTPase modulator of claim 9

40. The mutant GTPase of claim 22, wherein the GTPase modulator comprises an orthogonal GTPase modulator of claim 10.

41. The mutant GTPase of claim 22, wherein the GTPase modulator comprises an orthogonal GTPase modulator of claim 11.

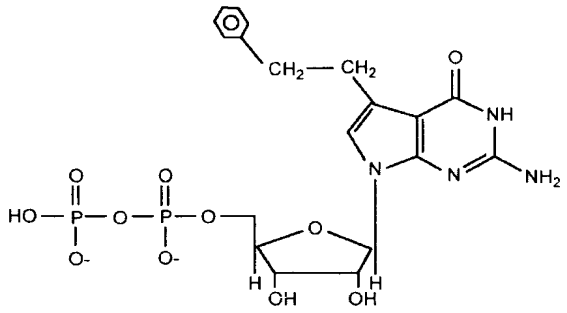
42. The mutant GTPase of claim 22, wherein the GTPase modulator comprises an orthogonal GTPase modulator of claim 12.

43. The mutant GTPase of claim 22, wherein the GTPase modulator comprises an orthogonal GTPase modulator of claim 13.

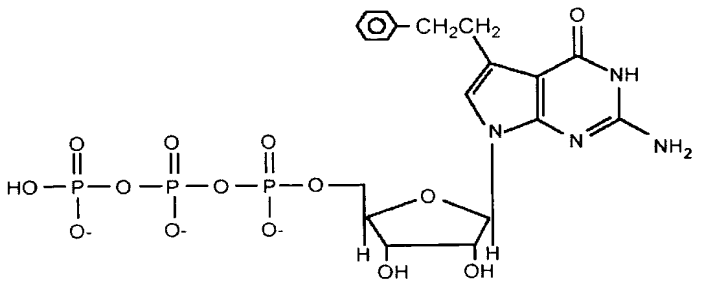
44. The mutant GTPase of claim 22, wherein the GTPase modulator comprises an orthogonal GTPase modulator of claim 14.

45. The mutant GTPase of claim 22, wherein the GTPase modulator comprises an orthogonal GTPase modulator of claim 15.

46. The mutant GTPase of claim 22, wherein the modulator is:



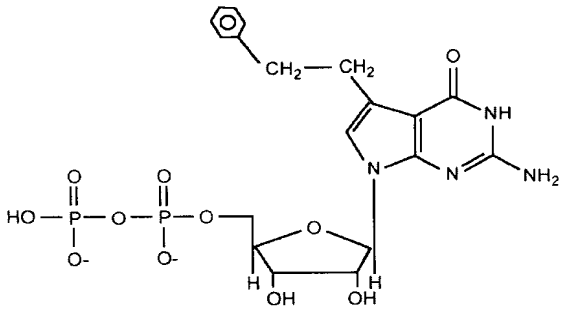

47. The mutant GTPase of claim 22, wherein the modulator is:



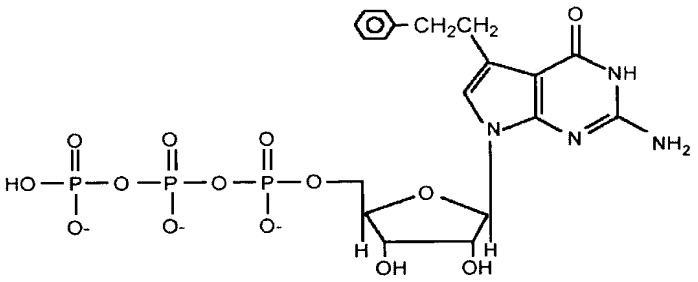
48. A complex comprising a mutant GTPase of claim 22 bound to a GTPase modulator that modulates the mutant GTPase but does not substantially modulate a corresponding wild-type GTPase.

49. The complex of claim 48, wherein the mutant GTPase comprises a double mutant having an alanine at first and second positions that correspond to L19 and N116 of H-Ras.

50. The complex of claim 48, wherein the GTPase modulator is:



51. The complex of claim 48, wherein the GTPase modulator is



52. The complex of claim 48, wherein the GTPase modulator comprises:

a) a sulfhydryl group that forms a disulfide linkage with a sulfhydryl group on the mutant GTPase, or

b) an electrophilic group that forms a covalent bond with a sulfhydryl group on themutant GTPase.

53. A host cell that comprises a mutant GTPase, which mutant GTPase comprises a non-native amino acid at one or more amino acid positions that correspond to N116, T144 and L19 of H-Ras, which mutant GTPase binds to a GTPase modulator that modulates the mutant GTPase but does not substantially modulate a corresponding wild-type GTPase.

54. The host cell of claim 53, wherein the host cell is selected from the group consisting of fibroblasts, myeloid leukemia cells, Raji cells, MIAPaCa-2 cells, PANC-1 cells, U-87 cells, U343 cells, U373 cells, EpH4 cells, human glioma cells, glioblastoma cells, and mammary epithelial cells.

55. The host cell of claim 53, wherein the host cells do not express a wild-type GTPase that corresponds to the mutant GTPase.

56. The host cell of claim 55, wherein a gene in the host cell that encodes the wild-type GTPase is disrupted.

57. The host cell of claim 53, wherein the host cell is present in an animal or a plant.

58. The host cell of claim 57, wherein the animal is a mammal.

59. A method of determining a GTPase function, the method comprising:
expressing at least one mutant GTPase in one or more host cells;
contacting the mutant GTPase with at least one GTPase modulator that binds to the mutant GTPase but does not substantially modulate a corresponding wild-type GTPase; and
detecting at least one result of applying the GTPase modulator to the cell, thereby determining the function of the GTPase.

60. The method of claim 59, wherein the mutant GTPase comprises a non-native amino acid at one or more amino acid positions that correspond to L19, N116 and T144 of H-Ras.

61. The method of claim 59, wherein the GTPase comprises one or more mutant Ras proteins.

62. The method of claim 59, wherein the host cells are selected from the group consisting of fibroblasts, myeloid leukemia cells, and Raji cells.

63. The method of claim 59, wherein the host cells do not express a wild-type GTPase that corresponds to the mutant GTPase.

64. The method of claim 63, wherein a gene that encodes the wild-type GTPase is disrupted in the host cell.

65. The method of claim 63, wherein the host cell is contacted with an antisense nucleic acid or an siRNA that inhibits expression of the corresponding wild-type GTPase but not the mutant GTPase.

66. The method of claim 59, wherein detecting comprises performing one or more assays to detect one or more functions of the mutant GTPase.

67. The method of claim 66, wherein the one or more assays comprise a GDP displacement assay.

68. The method of claim 59, wherein detecting further comprises determining one or more downstream response pathways affected by modulating the GTPase.

69. The method of claim 68, further comprising collecting data regarding the downstream response pathways and storing the data in at least one database.

70. The method of claim 59, wherein detecting comprises obtaining a gene expression profile of the cell in the presence and absence of the GTPase modulator to identify genes that are upregulated or downregulated in the presence of the GTPase modulator.

71. The method of claim 59, wherein contacting the mutant GTPase with the GTPase modulator comprises forming a covalent linkage between the GTPase modulator and an amino acid residue of the mutant GTPase, thereby modulating the activity of the GTPase.

72. The method of claim 71, wherein the mutant GTPase comprises a cysteine residue at one or more amino acid positions that correspond to L19, N116, or T144 of H-Ras; and

wherein the GTPase modulator comprises a sulfhydryl group or an electrophilic group.

73. The method of claim 59, wherein the GTPase modulator comprises at least one affinity label.

74. The method of claim 59, wherein the corresponding wild-type GTPase comprises Ras, and wherein the mutant GTPase has a decreased affinity for GTP and GDP.

75. The method of claim 59, wherein the GTPase modulator comprises a C-6 and N-7 substituted guanine moiety.

76. The method of claim 59, wherein contacting the mutant GTPase with the GTPase modulator further comprises forming a covalent linkage between the mutant GTPase and the GTPase modulator, leading to irreversible modulation of the GTPase.

77. The method of claim 59, wherein contacting the mutant GTPase with the GTPase modulator modulates binding of GTP or GDP to the mutant GTPase.

78. A method of modulating activity of a GTPase in a cell, the method comprising:

introducing into the cell a mutant GTPase that comprises a non-native amino acid at one or more amino acid positions that correspond to L19, N116 and T144 of H-Ras, which mutant GTPase binds to a GTPase modulator that modulates the mutant GTPase but does not substantially modulate a corresponding wild-type GTPase; and

contacting the mutant GTPase with the GTPase modulator, thereby competing with the wild-type GTPase for binding to one or more cellular effector molecule and reducing the activity of the GTPase in the cell.

79. The method of claim 78, wherein the cell is present in an animal.

80. The method of claim 78, further comprising:

administering an antisense nucleic acid or an siRNA that inhibits expression of the corresponding wild-type GTPase but not the mutant GTPase, thereby reducing or further reducing the GTPase activity in the cell.

81. The method of claim 78, wherein the GTPase modulator comprises a cell permeable compound, and wherein contacting the mutant GTPase with the GTPase modulator comprises providing a therapeutic composition of the GTPase modulator.

82. The method of claim 78, wherein the GTPase modulator comprises an inhibitor.

83. The method of claim 78, wherein the GTPase modulator comprises an activator.

84. A method of screening for proteins that specifically bind a GTPase, the method comprising:

a) providing a mutant GTPase, which mutant GTPase retains the effector specificity of a corresponding wild-type GTPase;

b) contacting the mutant GTPase with at least one orthogonal GTPase modulator, which GTPase modulator binds to the mutant GTPase but does not substantially inhibit or activate the corresponding wild-type GTPase, thereby providing a mutant GTPase-modulator complex;

c) contacting the mutant GTPase-modulator complex with at least one GTPase binding protein; and,

d) detecting the at least one GTPase binding protein.

85. The method of claim 84, wherein the binding protein comprises an effector of the GTPase.

86. The method of claim 84, comprising providing the mutant GTPase by providing a cell lysate comprising the mutant GTPase

87. The method of claim 84, comprising providing the mutant GTPase by expressing a polynucleotide sequence encoding the mutant GTPase in a cell.

88. The method of claim 84, wherein the mutant GTPase further comprises a sequence tag.

89. The method of claim 88, wherein the sequence tag comprises a GST- or a histidine-sequence tag.

90. The method of claim 88, wherein the mutant GTPase is bound to a solid substrate.

91. The method of claim 90, wherein the solid substrate comprises a bead.

92. The method of claim 84, wherein:

a) a dialyzed cell lysate comprising at least one orthogonal GTPase modulator is contacted with a mutant GTPase bound to a solid substrate, thereby providing a substrate-bound GTPase-modulator complex;

b) contacting the substrate-bound GTPase-modulator complex with at least one GTPase binding protein; and,

c) detecting the at least one GTPase binding protein.

93. The method of claim 92, further comprising:

eluting GTPase binding protein bound to the GTPase-modulator complex prior to detecting the at least one GTPase binding protein.

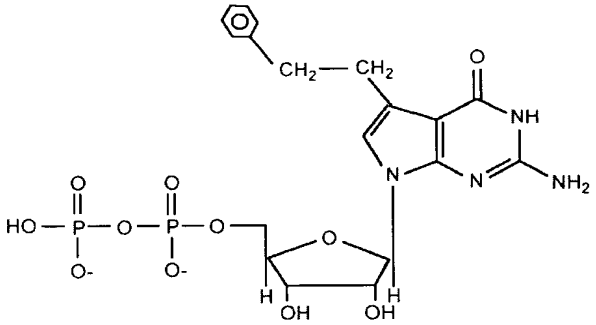
94. The method of claim 93, comprising eluting in a buffer comprising GTP.

95. The method of claim 93, comprising eluting in a buffer comprising GDP.

96. The method of claim 84, comprising detecting the at least one GTPase binding protein by two-dimensional electrophoresis.

97. The method of claim 84, further comprising identifying and/or isolating the at least one binding protein.

98. The method of claim 84, wherein the GTPase modulator is



99. The method of claim 84, wherein the GTPase modulator is